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Original Research Article

An Investigation of the Histological Effects of Diesel Contaminated Water on the Brain of Wistar Rats

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Abstract

Background: Water is an essential solvent and used daily by humans. Consumption of water contaminated with diesel has been shown to impact negatively on organs such as the brain, liver, spinal cord, kidney and others. The brain is a prime organ in the human body and responsible for general coordination and intelligence. Protecting the brain from harmful substances is necessary as this would mitigate neurodegenerative disorders which are obvious in our society. This study was carried out to determine the possible effects of diesel fuel on the histology of the brain of male albino wistar rate. ***Materials and Methods***: A total of 20 rates were used to carry out this research. The animals were grouped in 2 groups; groups A (Control) and B. Group A was administered with good feed and clean water. Group B was administered with feed and water contaminated with diesel for varying periods of time ranging from 1 to 5 weeks. At the end of the given periods the brains were harvested and histopathological investigations for alteration in brain tissues was carried out using routine tissue processing methods and H and E staining methods. ***Results and Discussion***: There was no histopathological alteration of brian tissues harvested from the control animals which were administered with clean water and uncontaminated feed. There was no alteration of brain tissues observed after week 2. The brain tissues harvested from animals whose feed and water were contaminated with diesel showed no histopathological changes when compared with that of the control (group A). A similar observation was made for weeks 3 through 5 for the animals in group B. ***Conclusion***: There were no observed effects of diesel contaminated water on the histology of the brain tissues of male albino wistar rats which may be due to the active protecting effect of the blood-brain barrier.
Keywords: Diesel, Rats, Brain, Water, Histology.

INTRODUCTION

Diesel is a complex mixture of chemicals (hydrocarbons) mainly obtained from the distillation of crude oil. The product is thought to be named after Dr. Rudolf Diesel, a German engineer who, in 1892, patented an oil-burning internal combustion engine [1].

There are no natural sources of diesel. Diesel is found in the environment as a result of accidental release from an industrial site or transport vehicle [1].

It is produced by blending several fractions of crude oil distillates with brand-specific chemical additives [1]. The actual chemical composition of diesel varies widely according to the geographical source of crude oil, but generally comprises C8-C21 aliphatic hydrocarbons (boiling range 160-360^{0}C) with up to 25% aromatic compounds. The (UK) technical terms for diesel are 'class A1 Fuel Oil' and 'Class A2 Fuel Oil' and refer to use in domestic and agricultural vehicles, respectively [3].

Like other fuels, the major use of diesel is in transportation to run cars, aircrafts and trains. It is also used to power generators for private and public use [3].

Toxicity occurs following ingestion, inhalation & skin absorption. Like most chemicals, the amount of diesel one is exposed to must be above a certain level to cause adverse health effects. A short, exposure to diesel will not normally cause any long-term health effects. Occasional skin exposure may lead to dermatitis (eczema). Breathing large quantities of diesel vapour or drinking diesel-based fluids is thought to cause non-specific signs and symptoms of poisoning such as dizziness, headache and vomiting. A severe form of lung damage called pneumonitis may occur if liquid diesel is inhaled directly onto the lungs, for example, whilst manually siphoning a tank or from inhaling vomit after swallowing diesel. This is why it is important not to make someone sick if they have swallowed diesel [4-6].

As diesel is a mixture of chemicals, there is no definitive Absorption, Distribution, Metabolism and Excretion (ADME) data.

Diesel accounted for all spillages resulting from road traffic incidents in the UK during 2003 and 42% of all significant (Environment Agency Category 1 or 2) pollution incidents for the same period [4-6].

Physicochemical Properties of Diesel

Diesel has low volatility and vapour pressure less than 1 mmHg. It is flammable and has a specific gravity of 0.82-0.95 at 15^0C (water = 1). Diesel does not mix with water but floats on it [10].

It may liberate irritating or toxic fumes during combustion and has a characteristic odour.

Immediate Signs or Symptoms of Acute Exposure

- Inhalation: induce headache, dizziness, drowsiness, incoordination and euphoria. Aspiration into the lungs causes pneumonitis with choking, coughing, wheeze, breathlessness, cyanosis and fever [10].
- Ingestion: Often no symptoms occur but there may be nausea, vomiting and occasionally diarrhea.
- Ocular exposure: This product is expected to be pH neutral but may be irritating to the eyes causing an immediate stinging and burning sensation with lachrymation. [10]
- Dermal exposure: Irritant. Drying and cracking due to defatting action. There may be transient pain with erythema, blistering and superficial burns [10].

Signs of ingesting diesel fuel through contaminated water include:

- Abdominal pains
- Vomiting
- Burning in the throat, noise, eyes, lips and tongue.

Environment Hazards of Sulfur

High levels of sulfur in diesel are harmful for the environment because they prevent the use of catalytic diesel particulate filters to control diesel particulate emissions, as well as more advanced technologies, such as nitrogen oxide (NO_2) adsorbers (still under development), to reduce emissions. Moreover, sulfur in the fuel is oxidized during combustion, producing sulfur dioxide and sulfur trioxide, which in presence of water rapidly convert to sulfuric acid, one of the chemical processes that result in acid rain (US Energy Information) [10].

Road Hazard

Petrodiesel spilled on a roadway poses a hazard to vehicles, due to its high evaporation temperature. After the light fractions have evaporated, a greasy slick is left on the road which can destabilize moving vehicles. Diesel spills severely reduce tire grip and traction, and have been implicated in many accidents. The loss of traction is similar to that encountered on black ice. Diesel slicks are especially dangerous for two-wheeled vehicles such as motorcycles [10].

Sources and Route of Human Exposure

One can be exposed to diesel through drinking or swimming in water that has been contaminated with diesel from a spill or leaking underground storage tank. Occupational exposure may potentially occur during manual filling or discharge operations within the petrochemical industry [14] repair or service of diesel engines or from practices where diesel is used as a cleaning agent or solvent [14]. Domestic exposure to diesel is uncommon, although limited skin exposure may occur whilst refuelling domestic vehicles and pulmonary exposure may result from aspiration of liquid during manual siphoning. Leakage of diesel onto hot engine manifolds may liberate a respirable aerosol of micrometer-sized diesel particles [14] Large-scale environmental contamination has occurred following the release of diesel from storage tanks and sea tankers and some concern has been expressed over health effects of vapour arising from contaminated soil [14].

The Brain

The brain is an organ that serves as the center of the nervous system in all vertebrate and most invertebrate animals—only a few invertebrates such as sponges, jellyfish, adult sea squirts and starfish do not have a brain, even if diffuse neural tissue is present. It is located in the head, usually close to the primary sensory organs for such senses as vision, hearing, balance, taste, and smell. The brain is the most complex organ in a vertebrate's body. In a typical human, the cerebral cortex (the largest part) is estimated to contain 15-33 billion neurons, each connected by synapses to several thousand other neurons. These neurons communicate with one another by means of long protoplasmic fibers called axons, which carry trains of signal pulses called action potentials to distant parts of the brain or body targeting specific recipient cells [15].

Physiologically, the function of the brain is to exert centralized control over the other organs of the body. The brain acts on the rest of the body both by generating patterns of muscle activity and by driving the secretion of chemicals called hormones. This centralized control allows rapid and coordinated responses to changes in the environment. Some basic types of responsiveness such as reflexes can be mediated by the spinal cord or peripheral ganglia, but sophisticated purposeful control of behaviour based on complex sensory input requires the information integrating capabilities of a centralized brain [15].

The functions of the brain depend on the ability of neurons to transmit electrochemical signals to other cells, and their ability to respond appropriately to electrochemical signals received from other cells. The

electrical properties of neurons are controlled by a wide variety of biochemical and metabolic processes, most notably the interactions between neurotransmitters and receptors that take place at synapses [15].

The human brain is the largest brain of all vertebrates relative to body size, it weighs about 3.3 pounds (1.5 kilograms) and makes up about 2 percent of a humans body weight. The cerebrum makes up 85 percent of the brains weight, it contains about 86 billion nerve cells (neurons), the 'gray matter' and contains billions of nerve fibers (axons and dendrites), the "white matter". These neurons are connected by trillions of connections, or synapses [15].

Blood-Brain Barrier

The blood-brain barrier (BBB) is a highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid (BECF) in the central nervous system (CNS). The blood—brain barrier is formed by brain endothelial cells, which are connected by tight junctions with an extremely high electrical resistivity of at least 0.1 [15-18]. The blood—brain barrier allows the passage of water, some gases, and lipid soluble molecules by passive diffusion, as well as the selective transport of molecules such as glucose and amino acids that are crucial to neural function. On the other hand, the blood-brain barrier may prevent the entry of lipophilic, potential neurotoxins by way of an active transport mechanism mediated by P-glycoprotein. Astrocytes are necessary to create the blood—brain barrier. A small number of regions in the brain, including the circumventricular organs (CVOs), do not have a blood-brain barrier [15-18].

The blood—brain barrier occurs along all capillaries and consists of tight junctions around the capillaries that do not exist in normal circulation [15-18].

Endothelial cells restrict the diffusion of microscopic objects (e.g., bacteria) and large or hydrophilic molecules into the cerebrospinal fluid (CSF), while allowing the diffusion of small hydrophobic molecules (02, C02, hormones) [15-18]. Cells of the barrier actively transport metabolic products such as glucose across the barrier with specific proteins [15-18]. This barrier also includes a thick basement membrane and astrocytic endfeet [15-18].

The blood—brain barrier acts very effectively to protect the brain from many common bacterial infections. Thus, infections of the brain are very rare. Infections of the brain that do occur are often very serious and difficult to treat. Antibodies are too large to cross the blood—brain barrier, and only certain antibiotics are able to pass [15-18]. In some cases a drug has to be administered directly into the cerebrospinal fluid (CSF) [15-18].

Brain Disorders

The brain is susceptible to many different disorders that strike at every stage of life. Developmental disorders such as autism and dyslexia first appear in early childhood. Psychiatric diseases such as depression and schizophrenia are typically diagnosed in teens or early adulthood, although their origins may lie much earlier in life. And, as we age we become increasingly susceptible to Alzheimer's disease, Parkinson's disease, stroke, and other diseases [21, 22].

Water is an essential solvent and used daily by humans. Consumption of water contaminated with diesel has been shown to impact negatively on organs such as the brain, liver, spinal cord, kidney and others. The brain is a prime organ in the human body and responsible for general coordination and intelligence. Protecting the brain from harmful substances is necessary as this would mitigate neurodegenerative disorders which are obvious in our society. Diesel is a common fuel utilized by vehicles and generators which makes it popular and pollution with it is unavoidable since it's a fuel that generates power and needed on a daily basis. When fuel oils burn, it burn partially thereby generating smoke and fine particles which floats in the air and easily get into the lungs through inhalation. Fumes from diesel oils resulting from leakage, exposure of storage tanks and open spill are also pollutants of the environment [21, 22].

There have been previous works on diabetes and Garcinia Kola reported by well known authors [2, 4-6, 11, 13, 15, 18, 21, 22].

Aim of the Study

This was aimed at investigating possible histological effects of diesel contaminated water on the brain tissues.

MATERIALS AND METHODS

Research Design

The study was experimental.

Sample Size and Sampling Technique

A total of 20 adult male albino wistar rats weighing about 200g each were randomly selected for the study.

Criteria for Subject Selection

Normal male wistar rats without any known deformities with relatively equal body weights.

Ethical Clearance

Ethical clearance was obtained from the Research Ethics Committee of the University of Port Harcourt, Nigeria.

Data Collection

The animals were kept in a regularly cleaned wooden wire netted cages. A period of two weeks was used for acclimatization in the holding facility. During this period, adequate attention was given to the animals to ensure that they are free from infection. Observations made within this period showed that animals were agile, fur texture and appetite for food and water was normal. The animals were fed with mice pellet and clean drinking water under the ambient temperature range of about 32°C-37°C.

The diesel used for this study was commercially purchased from NNPC petrol station located at Ahoada town, Rivers State, Nigeria. It is of the petro diesel variety. The rats were grouped into 2 groups, of 10 rats each.

Group A (control group) was exposed to feeds and clean water and Group B was exposed to feeds and diesel contaminated water for a period of 1 week, 3 weeks and 5 weeks respectively.

The rats in each group to be sacrificed were anaesthetized under mild anesthesia (chloroform). Brain tissues were harvested and immediately fixed.

Tissue preparation

Fixation

The experimental animal was decapitated, some brain tissues removed from the skull through the aid of the bone cracker. The harvested part was cut longitudinally into two equal halves and fixed for duration of 1hour in 10% formal saline. This was done to maintain the morphological integrity of the tissue.

Dehydration

This was done using ascending grades of alcohol ranging from 70% - 80% - 95% - three changes of absolute alcohol (100%).

Clearing

Clearing was done using three changes of xylene for one hour each to remove alcohol from the tissue and increase the refractive and optical index of the tissue.

Impregnation

This was done in molten paraffin wax of about 700 centigrade in order to remove xylene from the tissue and subsequently infiltrate the tissue pores and cavities to achieve good hardening.

Embedding

This is the next step after tissue processing and was done with L shaped rods and metal plates block filled molten paraffin wax and then the tissue is placed immediately with forceps. The surface to be cut facing downwards, the paraffin was allowed to cool so that the thin scum of solid paraffin is formed on the bottom of the metal blocks after which it was immersed in water. When the paraffin was solidified, the L metal blocks were removed from the paraffin block and the tissue formed uniformly ready for sectioning.

Sectioning

This was done with a microtome to obtain uniform sections at 5 microns. Sections were floated with a water bath and picked with a frosted glass slide. Immediately, sections were placed on a hot plate for about 30minutes.

Staining

The staining was done using Haematoxylin and Eosin stains (H and E). Haematoxylin is a dye that stains the nucleus violet or blue and eosin stains the cytoplasm pink.

RESULTS

Physical and Behavioural Changes

At the beginning of the experiment, all the animals were healthy and agile. During the two weeks of acclimatization, their stools were normal. On administration of diesel, varying gradations of toxicity were observed. Generally, the signs of toxicity observed included: Scattered hairs, muscle tremor, lacrimation, diarrhea, tachycardia, salivation, weakness, frequent urination.

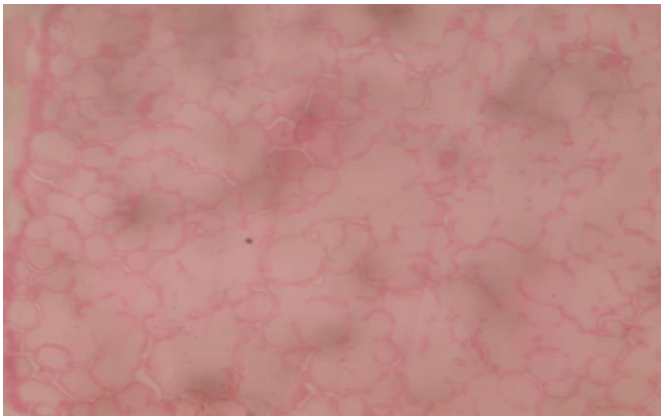

Fig-1: Brain Tissues in Control Group at week 5

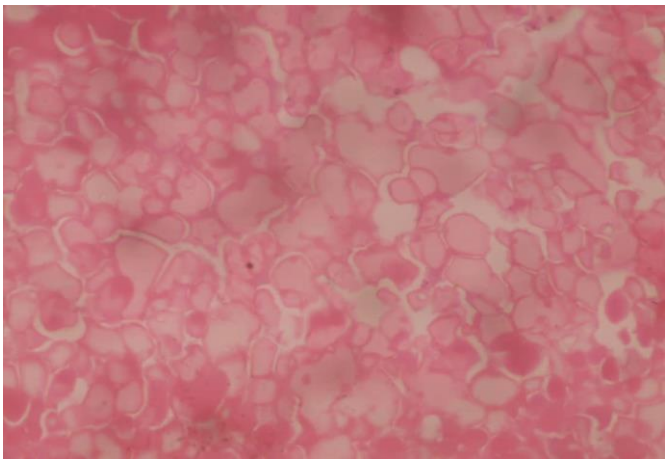

Fig-2: Brain Tissues in Experimental Group at week 5

DISCUSSIONS

Physical observations of the animals in the different groups showed that the group B animals did not drink the water during week 1 due to diesel contamination. The smell irritated the animals. From week 2 the animals in group B started drinking the water contaminated with diesel (hesitating) presumably due to dehydration. From weeks 3 through 5, the animals in group B drank the contaminated water without hesitation. They got used to it.

Histopathological findings for the control groups from week 1 through week 5 showed no changes. There was no histopathological alteration of brain tissues harvested from the control animals which were administered with clean water and uncontaminated feed. This result negates the reports of similar studies done by other researchers [13-21].

A comparison of the histopathological findings of group 2 with the control group showed no changes. There was no alteration of brain tissues observed after week 3. The brain tissues harvested from animals whose feed and water were contaminated with diesel showed no histopathological changes when compared with that of the control (group A). A similar observation was made for weeks 1 through 5 for the animals in group B. This result contradicts the reports of similar studies done by other researchers [13-21].

The possible reason for this observation could be the blood-brain barrier that acts very effectively to protect the brain against the diesel fuel. Thus, infections of the brain are very rare. Antibodies are too large to cross the blood—brain barrier, and only certain antibiotics are able to pass [4-6]. In some cases a drug has to be administered directly into the cerebrospinal fluid (CS F) [4-6].

There are also some biochemical poisons that are made up of large molecules that are too big to pass through the blood—brain barrier. This was especially important in more primitive times when people often ate contaminated food. Neurotoxins such as botulinum in the food might affect peripheral nerves, but the blood—brain barrier can often prevent such toxins from reaching the central nervous system, where they could cause serious or fatal damage [18].

CONCLUSION

There were no observed effects of diesel contaminated water on the histology of the brain tissues of male albino wistar rats which may be due to the active protecting effect of the blood-brain barrier.

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Conflict of interest

We write to state that there is no conflict of interest.

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Author's contribution

We write to state that both authors have contributed significantly, and that all authors are in agreement with the contents of the manuscript. 'Author A' (Josiah S. Hart) designed the study and protocol, 'reviewed the design, protocol and examined the intellectual content and 'Author B' (John Nwolim Paul) wrote the first draft of the manuscript, managed the literature search and managed the analyses of the study. All authors read and approved the final manuscript.

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